Fungi: toxic killers or unavoidable nuisances?

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Objectives: This discussion is focused on the many roles of fungi in human health, and also to put the mycotoxin literature into perspective.

Data Sources: Data are derived from the literature referenced in PubMed from the National Library of Medicine, earlier references in the authors' reprint collection, and ongoing research. Studies for review were either selected from the peer-reviewed literature or from standard texts that are well recognized in the field.

Results: The review yielded many studies of the role of fungi in allergic disease, but none that systematically documented such a role for mycotoxins or fungal volatiles. Many case studies were found, but none of these unequivocally document a cause/effect relationship between mycotoxin exposure by inhalation and human disease in residential, school, or office settings.

Conclusions: The review led to the conclusion that that the primary result from fungal exposure is allergic disease, and that the evidence for inhalation disease resulting from mycotoxin exposure in residential and office settings is extremely weak.

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INTRODUCTION

In the search for inexpensive shelter, we have developed indoor environments that are conducive to fungal contamination. Although active fungal growth indoors is usually inappropriate and should be controlled, evaluating specific health risks associated with such growth remains a challenge. People have become concerned about the health effects of mycotoxins out of proportion to currently estimated risk. This review of the indoor and mycotoxin literature is designed to present the state of knowledge as it is today, and to put fungal exposure into perspective.

THE FUNGI

Fungi are filamentous organisms that absorb food from the environment after external digestion (absorptive nutrition). The cell walls of the fungi are formed of chitin (acetylglucosamine polymers), β-(1–3)-D-glucans, polysaccharides, and mucopolysaccharides that carry some antigenic specificity, waxes, and pigments of various types. The most common pigment in fungal cell walls is melanin, which is present, as in humans, to protect against ultraviolet.[1] The glucans in the fungal cell wall are endotoxin-like substances that may be irritating and stimulate the immune system.[2] During growth, the fungi release enzymes that can be allergenic and inflammatory into the environment to digest food to a soluble form for adsorption. In the digestion process, new enzymes and secondary metabolites are released that can also be allergenic (eg, the enzymes), irritating (eg, volatile metabolites), or toxic for some forms of life (eg, mycotoxins, antibiotics). A very few of the fungi can invade and grow in the human body causing infectious disease. In some cases, this invasion may be facilitated by some of the mycotoxins.

All fungi probably produce allergenic substances. However, of the hundreds of thousands of different kinds of fungi, only a very few have been tested for allergenicity. Although not the focus of this paper, it is important to remember that fungal allergy is common (as many as 10% of the entire population and at least 40% of asthmatic patients may be fungal-sensitive), and those with fungal sensitivity may have more serious disease than those with other sensitivities. However, inhalation mycotoxicosis is rare, even in agricultural environments where exposures to fungal spores can be intense.[3]

MYCOTOXINS

All fungi probably produce mycotoxins, which are secondary metabolites that the fungi produce during the process of metabolizing food. They are considered secondary metabolites because they do not nourish the fungi and have no apparent physiologic function in the fungus. However, they probably do contribute in the battle for food by helping to reduce competition from other organisms.[1]

The mycotoxins are of relatively low molecular weight and generally considered nonvolatile. Table 1 lists some of these toxins and some of their fungal sources.

Virtually all information we have on the health effects in Table 1 comes principally from either animal or (less commonly) human ingestion data, and from laboratory animal exposures. Only for aflatoxin is there limited epidemiologic data for human respiratory exposures.[4,5] So far, no study in humans unequivocally documents a connection between inhalation of mycotoxins and disease. Although mycotoxins clearly could cause reported health effects, each case has failed to demonstrate that a sufficient dose was or even could have been achieved under the given conditions. In some cases this does not mean that the exposures did not occur; only that the data to prove exposure are missing. In other cases, conclusions have been drawn in the presence of data casting serious doubt on the potential for exposure.

EXPOSURE CONSIDERATIONS

Exposure, then, is usually the missing link in case studies that suggest an association between the presence of toxigenic fungi and human disease. Exposure involves the presence of the toxin, transfer of the toxin from the source to

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Table 1. Some Common Fungi, Mycotoxins, and Health Effects from Ingestion, Dermal, or Inhalation Exposure[1,19,20]

Fungus	Mycotoxin	Possible health effect
Alternaria alternata, Phoma sorghina	Tenuazoic acid	Nephro- & hepatotoxic, hemorrhagic
Aspergillus flavus, A. parasiticus	Aflatoxins	Mutagenic, carcinogenic, hepatotoxic
Aspergillus fumigatus	Fumitremorgens Gliotoxin	Tremorgenic, Cytotoxic
Aspergillus ochraceus, Phoma viridicatum, Trichophyton verrucosum	Ochratoxin A	Nephro- & hepatotoxic, carcinogenic
Aspergillus nidulans, A. versicolor, Cochliobolus sativus	Sterigmatocystin	Hepatotoxic, carcinogenic
Cladosporium sp.	Epicladosporic acid	Immunosuppressive
Fusarium poae, F. sporotrichioides	T-2 toxin	Hemorrhagic, immunosuppressive, causes nausea, vomiting
Fusarium moniliforme	Fumonisins	Neuro-, hepato-, nephrotoxic, carcinogenic
Penicillium griseofulvum, P. viridicatum	Griseofulvin	Tumor & teratogenic, hepatotoxic
Penicillium expansum	Patulin Roquefortine C Citrinin	Nephrotoxic, carcinogenic
Pithomyces chartarum	Sporidesmin Phyllоerythrin	Hepatotoxic; causes photosensitization, eczema
Stachybotrys chartarum (atra)	Satratoxins Verrucarins, Roridins	Inflammatory, immunosuppressive, cause dermatitis, hemotoxic, hemorrhagic

the body, and release or distribution of the toxin within the body in amounts sufficient to cause the observed symptoms. Obviously, the observed symptoms must have a logical cause/effect relationship with the toxin. The generalized pathway for tracking exposure and subsequent response is outlined in Table 2. This particular example is an aerobiologic pathway, although the principle certainly can be applied to other routes of exposure.

Pathways for Exposure

Assuming that, for example, *Stachybotrys chartarum* is present in the case environment, we can follow the exposure pathway to study the chances that the toxins produced by this infamous organism are likely to be causing disease.

Source Factors

Amounts of Fungal Growth. Obviously, the more fungal growth there is, the more likely there will be exposure, given that the growth is in a site where release and aerosolization of spores are possible. There are no good guidelines for the amount of growth that is likely (or not) to result in exposure for any kind of fungus.

Amounts of Toxin Produced by the Fungus. The amount of toxin produced by a given fungal colony depends on: 1) the species of fungus; 2) the genetic pattern of the particular strain of the species; 3) the length of time it has been growing; 4) the kind of food available; 5) the amount of water available; 6) the temperature; 7) light amounts and wavelengths; 8) presence or absence of competition; and other unknown factors. These factors are all interactive. Thus, one speaks of a particularly toxigenic strain of a particular species, or a particular food source that stimulates mycotoxin production. In each case, these factors have to be determined by actually measuring toxin content of the fungus as it grows on the wall. Conditions in culture may produce irrelevant results.[6]

Particle Release Factors. Small air movements readily disperse some fungal spores whereas others require some kind of mechanical abrasion. *Penicillium* and *Aspergillus* spores are readily airborne, whereas *S. chartarum* spores require mechanical disturbance. Disturbance may come with brushing against or washing the growth, or with renovating activities. If growth occurs in closed spaces, a pathway into the breathing zone must be extrapolated, and concentrations must be estimated with a consideration of the reduction related to release from the enclosure.

Aerosol Factors

Concentration Patterns of the Aerosol. Fungal spore aerosols always vary over time. In the case of *Stachybotrys*, few spores are found consistently in the air even in spaces with extensive, active growth.[7] Even in the notorious Cleveland homes where *Stachybotrys* exposure continues to be blamed for infant deaths, levels of *Stachybotrys*

Table 2. A Generalization of the Aerobiologic Pathway Leading to Inhalation Exposure

Source factors	→	Aerosol factors	→	Exposure factors	→	Response factors
The organisms		Composition of aerosols		Time spent in aerosol		Dose reaching appropriate organ
Populations, their interactions and dynamics		Particle size distribution		Breathing rate		
		Dispersion		Particle deposition sites		Dose needed for effect
Chemistry, physiology, biology of the organisms		Biologic decay		Clearance rates		Metabolism of toxin
		Physical decay		Metabolic destruction of toxin		Human susceptibility factors
Particle release factors		Patterns of aerosol concentration				

spores were nearly undetectable (<10 spores per cubic meter of air).[8] This is, at least in part, because *Stachybotrys* spores are relatively large and sticky, and tend to settle quite quickly.

Amounts of Toxin in Exposure Units. Spores are probably the most common inhalation exposure unit for mycotoxins. Table 3 presents some of the very limited data available on amounts of particular toxins per unit of spores, expressed as nanograms of toxin per gram, and as nanograms of toxin per one million spores. Thus, if the required dose of a toxin to cause an acute effect were 0.1 mg/kg body weight and there were 10 ng toxin/one million spores (10^6), then it would take an exposure in the body of $(1 \times 10^5)/(1 \times 10^{-5}) = 10^{10}$ spores kg. Although these are fictitious figures, they are not unreasonable estimates of the requirements for an acute toxic dose per kilogram of body weight.

Table 3. Toxins from Spores of Several Fungal Species Cultivated on laboratory media; Quantification by High-Pressure Liquid Chromatography or Thin-Layer Chromatography[21,22]

Fungus	Toxin(s)	ng/g Spores	ng/10^6 Spores
Aspergillus fumigatus	Fumigaclavine C	930,000	9.890
*Aspergillus fumigatus**	Fumitremorgen B + verruculogen	—	6–80
Aspergillus niger	Aurasperone C	460,000	0.114
Aspergillus parasiticus	Aflatoxin B1	16,600	0.976
Penicillium oxalicum	Secalonic acid D	1,890	0.025
Aspergillus parasiticus, (mutant Nor-1)	Norsolorinic acid	280	0.023

Table 4. Inflammatory and Cytotoxic Effects of Four Fungal Species Grown on Six Types of Plasterboard[9]

	NO >10 nmol	IL-6 >1500 pg	IL-1β >600 pg	TNFα >2500 pg	Cytotoxicity >50% cell death
S. californicus	10	12	7	12	8
P. spinulosum	0	2	0	10	6
A. versicolor	0	6	2	8	12
S. chartarum	0	6	0	9	9

Number or percent of 12 samples (6 plasterboards with replicates) producing effects shown)

Exposure Factors

Time Spent in the Aerosol. Time is always one of the crucial factors in the transmission of inhalational disease. The longer the time spent in an aerosol, the more likely a sufficient number of particles is to be inhaled. Thus, the number of particles available to enter the respiratory tract is equal to time spent in the aerosol times the aerosol concentration over the same time period. If you divided the fictitious number generated above by 10 hours, it would take 10^9 spores per hour to make the effect possible (assuming that exposure is cumulative).

Amounts of Toxin Reaching Affected Organ System. This is the product of the number of spores retained in the respiratory tract, the amount of toxin per spore, the rate of toxin release from the spore, and the rate that the toxin is removed from the site or destroyed. This series of factors is unknown for the mycotoxins. However, some steps can be extrapolated from information on particle size and the range of reported concentrations of toxin per particle. Because of their relatively large size, many of the *Stachybotrys* spores inhaled are likely to deposit in the upper airways. This may be an important site for exposure, but would lead to symptoms first at the impacted site, then systemic symptoms, providing the dose was adequate. A few spores would enter the lower airways. This is the number of particles and the amounts of toxin that would have to be considered in calculating lung dose.

Response Factors

Effects. There is now quite a large body of data on the effects of the *Stachybotrys* toxins in animals and a few studies of other toxins. In addition, effects of spores of several other toxigenic fungi have been studied on isolated macrophages and other cells. One study compared the effects of spores of *Streptomyces californicus*, *Penicillium spinulosum*, *Aspergillus versicolor*, and *S. chartarum* grown on different types of wallboard on the production of inflammatory mediators and cytotoxicity in macrophages.

These data point out the wide variability of toxic effects among different species, these effects for each species on different types of wallboard, and the fact that the strain of *Stachybotrys* used was among the least inflammatory and toxic.[9] These data are summarized in Table 4. Although other strains of *S. chartarum* could have been much more effective, it is extremely important not to forget that *Stachybotrys* is not the only important fungus that can grow in indoor environments.

Amounts of Toxins Required for Effect. Studies of the acute toxic effects of *Stachybotrys* toxins have required very large doses of inhaled or instilled toxin.[10,11] The studies mostly use extracted toxin instead of intact spores so that doses can be more easily controlled.[12] Realistic chronic exposures via inhalation have not been studied for any animal and certainly not for humans. Laboratory animal data are extremely useful for determining cause/effect relationships, but it is difficult to extrapolate to the human situation.[13,14] Specific animals can be more or less sensitive to a toxin than humans, and exposure pathways into the respiratory tract may be quite different.

EXAMPLES OF EVIDENCE FOR HUMAN HEALTH EFFECTS

None of the human health effects studies have documented all exposure pathway steps, although some have come quite close. The two key cases

Table 5. Two Prominent Publications on Toxicity of *Stachybotrys* Mapped on the Aerobiologic Pathway

Author	Source measure	Aerosol measure	Exposure measure	Response	Authors' conclusions	Comments
[23]Croft et al, 1986	Documented toxin in reservoirs	High volume air samples tested for trichothecene toxins (positive)	Documented toxin in sources with logical pathway for exposure	Cold/flu symp., sore throats, dermatitis	Hypothesis for trichothecenes causing illness is plausible but not proven	Allergic alveolitis symptoms were present dose/response data not provided
[24]Montana, 1997; [25]Sorenson et al, 1996; [26]Etzel et al, 1998	Toxic isolates of *S. chartarum* recovered from reservoirs	Cassette sampling revealed very low to zero concentrations (<10/m^3 over all studies) of *S. chartarum* spores	Houses were moldy, but no logical pathway for exposure demonstrated	Hemosiderosis	*Stachybotrys* toxin exposure over time led to fragility of infant capillaries and hemosiderosis	No logical pathway; no documentation of spores in air; no explanation of how sufficient dose entered lungs of babies; epidem. studies flawed; smoking a greater risk factor with approximately the same *P* value.

that have stimulated concern are summarized in Table 5 with respect to the exposure/response pathway.

Other studies that claim to make the association between *Stachybotrys* exposure and either hemosiderosis or other symptoms have similar flaws. In general, most ignore other factors in the environment, including the presence of other fungi in much greater concentrations than *Stachybotrys*; also, they generally do not consider whether or not an adequate dose of any mycotoxin could have reached the patient. It may be that fungal growth in buildings is a hazard, and, until proven otherwise, should be considered so. However, the evidence at this point is wholly inadequate, except for the connections that have been made between fungal exposure and allergic disease.

Other Health Effects (Excluding Allergic Disease)

There is some evidence that damp, moldy home environments contribute to an excess of lower respiratory illness events in occupant children.[15] These effects are separate from asthma, and do not bear the usual correlates as do respiratory allergies. Very recent data from our laboratories indicate that exposure to fungi may lead to an increased risk of lower respiratory illness in babies.

Several studies have implicated glucans as causal factors in the "sick building syndrome," or, as it is more reasonably called, "building-related nonspecific symptoms."[2] Fungal glucan has been shown to have some effects similar to those of endotoxin, and, in high enough concentration, could cause some irritation and inflammation.[16,17] However, the studies claiming cause/effect relationships do not meet the criteria for exposure/response as outlined by the aerobiologic pathway. Most are epidemiologic studies that demonstrate a weak relationship between glucan measurements in dust and some symptoms. However, there is no reason to think that it is the glucan itself that is causing the symptoms. The glucan levels may be acting as a surrogate for some other agents.

Other potentially toxic materials that are released by fungi are a variety of volatile organic compounds (MVOCs).[6,18] The odors associated with fungal growth are the result of the mixture of MVOCs produced by the fungi growing in the environment. As for the mycotoxins, MVOCs are essentially secondary metabolites that may aid in the competition battle. Also similar to the mycotoxins, their production is dependent on growth conditions. To date, no clear evidence supports a relationship between exposure to fungal volatiles and any health outcome, primarily because the volatiles are produced in such minute concentrations.[18] Even for *Stachybotrys*, one study has documented that in the absence of spore exposure, *Stachybotrys* growth does not cause acute respiratory effects in mice.[6]

AVOIDANCE AND ADVICE

News media can not attract audiences with messages that are not dramatic. "Babies dying of black mold exposure" is a much more exciting headline than "[B]abies dying of unknown causes." News stories tend to exaggerate conclusions that are exciting and underplay the problems with the data. The fact that a mold is growing in a home is not good evidence for exposure of any kind, and certainly not evidence of danger. A pathway for appropriate exposure must be documented. Because it is rarely airborne, *Stachybotrys* is probably less of an exposure problem than other fungi.

It is probably true that for any outcome but cancer, massive exposures to spores containing relatively high levels of toxins would be necessary to induce illness. This means levels *in excess of* 10^6 per cubic meter of air for short periods and constant exposures to lev-

els >1,000 toxin-containing spores per cubic meter for many days. These types of exposure are extremely rare and occur primarily in agricultural situations. Even for cancer, consistent exposure to levels higher than in the vast majority of homes and office/school workplaces would be necessary. These effects have only marginally been documented in agricultural situations. In general, then, one can reassure patients that the symptoms they are experiencing, although real, are probably not associated with mycotoxin exposure. With the mycotoxin issue set aside, one can then proceed to a more likely diagnosis.

Because living with mold is uncomfortable (unsightly growth, odors), dangerous for asthmatic patients, and potentially a problem for young children with respect to lower respiratory tract illness, fungal growth in homes and schools should be minimized. The absolutely essential step for controlling fungal growth is to remove water from the environment. No other remediation effort will have lasting effects.

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